Prevalence of pancreatic, hepatic and renal microscopic lesions in post-mortem samples from Cavalier King Charles Spaniels

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Structured Summary

Objectives: To describe the prevalence of pancreatic, hepatic and renal microscopic lesions in post-mortem samples from Cavalier King Charles Spaniels (CKCS) presented to a UK post-mortem collection scheme.

Methods: Histopathology was performed on the organs of interest and the prevalence of microscopic lesions described, this was related back to the clinical signs shown ante-mortem.

Results: Evidence of chronic pancreatitis was seen in 51.9% of the cases, and age correlated with severity of disease, suggesting that chronic pancreatitis is a progressive condition. Evidence of renal lesions was present in 52.2% of cases, most commonly inflammatory disease. The rate of ante-mortem diagnosis was low for both pancreatic and renal disease, at 25% and 16.7% respectively. Primary hepatic lesions were present in only 11.1% of cases, but secondary hepatic lesions were more common and were present in 64.8%.

Clinical Significance: Pancreatic and renal lesions are common in Cavalier King Charles Spaniels and clinicians should be aware of this when presented with clinical cases, they have similar rates of hepatic disease as the general population.

Keywords: Chronic pancreatitis; Cavalier King Charles Spaniel; Renal; Hepatic; Pathology

Introduction

The Cavalier King Charles Spaniel (CKCS) is a common breed in the United Kingdom. The breed is commonly reported to suffer from chronic mitral valve disease (Beardow and Buchanan 1993) and syringomyelia (Rusbridge *et al.* 2000), however less information exists on other conditions, such as pancreatic, renal and hepatic disease, although there is a suspicion of an increased prevalence of chronic pancreatitis in the breed (Watson *et al.* 2007).

The Cavalier collection scheme* was initially started by motivated cavalier owners and breeders, in order to obtain post-mortem samples of the central nervous system (CNS) from CKCS which had had MRI prior to death to gain a better understanding of syringomyelia. It was then extended to include samples of mitral valve for further studies of chronic valvular disease. More recently, as increasing recognition of other diseases developed, the scheme was extended again to include pancreatic, hepatic and renal samples.

Pancreatic, renal and hepatic diseases can be difficult to diagnose ante-mortem, due to the non-specific nature of clinical signs, a lack of highly sensitive diagnostic tests and variable disease progression. They can, however, cause significant morbidity in affected dogs and so an indication of prevalence would be useful to help guide clinicians to undertake appropriate diagnostic tests in living animals.

This study aimed to assess the prevalence of pancreatic, renal and hepatic lesions in a population of CKCS presented to a post-mortem collection scheme and to relate these back to the clinical history of those dogs. In addition, the study also aimed to assess whether there was any statistically significant association between the different abnormalities detected.

*http://www.thecavaliercollectionscheme.org

Materials and methods

Sections of pancreas, liver and kidney were taken from the dogs presented to the Cavalier collection scheme by their owners with fully informed consent. In a small number of dogs samples were only available for one or two of these organs. Some of these cases underwent a full post-mortem examination at one of the participating centres, whilst some cases had samples of the appropriate organs collected by veterinary surgeons in first-opinion practice in the UK. Full instructions were sent to the veterinary surgeons to ensure consistent sample collection (supplementary file 1). CNS samples were only taken if the dog had a post-mortem examination at an appropriate centre.

Each dog's case also required completion of the submission form for the collection scheme, containing questions about relevant medical history. Some cases also had a full clinical history available. Medical history required included age and presence or absence of neurological, cardiovascular or gastrointestinal signs and any concurrent diseases (supplementary file 2 and 3).

All tissue samples were fixed in 10% neutral buffered formalin solution before being embedded in paraffin. The paraffin sections (5μm) were then stained with hematoxylin and eosin (H & E) and reviewed by two of the authors.

Pancreatic lesions were categorised as previously published (Newman *et al.* 2006, Watson *et al.* 2007). Chronic pancreatitis was defined as a lymphocytic or mixed inflammatory infiltrate (clusters of lymphocytes, plasma cells, macrophages, neutrophils in combination with tissue changes) with or without fibrosis, with disruption of the normal pancreatic architecture. All of the pancreas sections were allocated to one of the following categories based on Newman *et al* 2006:

a) No evidence of chronic pancreatitis. Scored as 0.

b) Mild chronic pancreatitis: one or two small foci of lymphocytic infiltrate and mild fibrosis in the section examined occupying less than 10% of the area. Scored as 1.
c) Moderate chronic pancreatitis: multi-focal areas of fibrosis and lymphocytes in the section examined but involving less than 40 per cent of area. Scored as 2.
d) Marked chronic pancreatitis: multi-focal areas of marked fibrosis and lymphocytic inflammation producing gross distortion of the normal architecture and affecting >40% of tissue. Scored as 3.
e) "End-stage" was regarded as virtually no normal acinar tissue remaining. Scored as 4.

The liver lesions were assessed according to the WSAVA standards for liver histopathology (Rothuizen 2006) and allocated to one of the following categories:

a) No evidence of hepatic disease.
b) Primary hepatic disease.
    I. Chronic hepatitis: hepatocellular apoptosis or necrosis with an inflammatory infiltrate (mononuclear or mixed) and varying degrees of fibrosis.
    II. Acute hepatitis: a combination of acute inflammation, hepatocellular apoptosis and necrosis, which may be accompanied by regeneration.
    III. Neoplasia.
c) Secondary hepatic disease
    I. Hepatic congestion: engorgement and dilation of the hepatic veins and centrilobular sinusoids, which may be accompanied by dilated lymphatics and centrilobular perivenular fibrosis.
    II. Hepatic vacuolation: hydropic change or fatty change.
    III. Reactive hepatitis: inflammatory infiltrate in the portal areas or parenchyma with no hepatocellular necrosis.

Renal lesions were assessed into the following categories (Macdougall *et al.* 1986):

a) Normal
b) Inflammatory disease
    I. Glomerulonephritis: proliferation of the endothelial and epithelial cells of the glomerular capillary with thickening of the glomerular basement membrane.
    II. Interstitial Nephritis: a mixed inflammatory infiltrate of the interstitium
c) Other abnormalities.

Dogs were excluded if neither a complete clinical history nor a complete submission form was available. Some cases did not have samples available from each organ.

Dogs were considered to have had clinical signs that may have been consistent with chronic pancreatitis if they had frequently reported episodes of gastrointestinal signs (vomiting, diarrhoea, inappetence) or abdominal pain (Watson 2012). A clinical diagnosis of renal disease was based on the identification of azotaemia, in the face of poorly concentrated urine (Lees 2004).

Prevalence of microscopic lesions were calculated for each organ and compared to the prevalence of clinical signs.. Relative risk was calculated for having renal disease together with chronic pancreatitis. It was considered significantly increased only if the risk and 95% confidence interval were greater than 1. Dogs with and without pancreatic lesions were compared for any significant differences in signalment. To document any increase in severity of chronic pancreatitis with age, Kendall's Tau rank correlation coefficient was used to compare age with chronic pancreatitis score.

Results

Fifty-nine dogs were initially included in the study, five were subsequently excluded due to unavailable histopathology (two) or unavailable clinical history (three), leaving 54 dogs for analysis.

The dogs' age ranged from 3 to 16 years (mean 10.4 years, median 10.9 years) and from 6 to 15.7kg in weight (mean 9.3kg, median 9.3kg). Dogs were euthanased or died for a number of reasons, including congestive heart failure (15), syringomyelia (10), other neurological disorders (11, most commonly degenerative myelopathy or seizures), neoplasia (6), renal disease (3), old age (3), liver disease (2), pancreatic disease (2), and 1 each of severe anaemia and aggression.

Eighteen dogs underwent a full post-mortem examination, the remainder had the relevant organs sampled by veterinary surgeons in practice. Twelve dogs also had histopathology performed on the CNS, these have been reported as part of a previous study (Hu *et al.* 2012).

Twenty-four dogs (44.4%) were being treated for cardiac disease at the time of death with a number of medications including one or more of the following list: frusemide, pimobendan, spironolactone, benazepril, torsemide and digoxin. Twenty-five dogs had an ante-mortem diagnosis of syringomyelia, some of which were receiving medical therapy including one or more of the following drugs: frusemide, gabapentin, prednisolone, and non-steroidal anti-inflammatory drugs.

The prevalence of microscopic lesions, and association with clinical signs, is described in tables 1-3. Examples of some of the most common abnormalities are demonstrated in figures 1-5 (pancreatic disease), and figure 6 (renal disease).

There was a weak but significant positive correlation between age and severity of chronic pancreatitis (tau_b correlation coefficient = 0.3; $p = 0.003$).

When considering inflammatory diseases, 8 dogs had both chronic pancreatitis and renal disease, 3 dogs had chronic pancreatitis and inflammatory liver disease and 1 dog had chronic pancreatitis, renal disease and inflammatory liver disease.

There was no increase in the relative risk of glomerulonephritis or interstitial nephritis in dogs with chronic pancreatitis (relative risk 0.8250, 95% CI 0.414 to 1.645, *P*=0.58). There was also no increased risk of vacuolar hepatopathy in dogs with chronic pancreatitis (relative risk 0.6845, 95% CI 0.364 to 1.289, *P*=0.24). There was no increased relative risk of hepatic congestion in dogs with cardiac disease (relative risk 0.9900, 95% CI 0.508 to 1.928, *P*=0.976). Whilst the relative risk of inflammatory kidney disease was increased in dogs with cardiac disease (relative risk 1.3228, 95% CI 0.664 to 2.634, *P*=0.43), the confidence interval falls below 1 so it is unclear how significant the association is.

Discussion

This is the first study to examine the prevalence of histopathologically confirmed pancreatitis, renal and liver disease in a large population of CKCS examined at post-mortem. Whilst the prevalence of pancreatitis in the dog has been examined in previous studies (Newman *et al.* 2004, Watson *et al.* 2007), this is the first study to focus on only one breed. This study shows the prevalence of chronic pancreatitis in the CKCS is 51.9%, which is greater than the 34% reported in a large study looking at post-mortem samples from a variety of breeds (Watson *et al.* 2007). That study included 6 CKCS, all of which had evidence of chronic pancreatitis. The prevalence of renal disease was also very high in this study at 52.2% and yet the proportion of dogs with ante-mortem diagnosis of either chronic pancreatitis or renal disease was very small. The prevalence of hepatic lesions is very similar to that reported in the general population (Watson *at al.* 2010b).

Chronic pancreatitis is suspected to be an under-diagnosed disease, mainly due to the vague nature of the clinical signs (Watson 2004) and this study supports that. We found that only 24% of the cases with histological evidence of chronic pancreatitis had been diagnosed ante-mortem. This may be because the clinical signs were very intermittent, interpreted to represent an alternative disease or because the clinical signs were genuinely so subtle. However, chronic pancreatitis is known to be an important cause of chronic pain in dogs (Watson *et al.* 2010a, Bostrom *et al.* 2013) and in humans (Bouwense *et al.* 2013). The pain in humans results in a significant upregulation of central nociception, which is more marked the more severe the disease is (Bouwense *et al.* 2013). It is possible that cavaliers with chronic pancreatitis suffer from chronic pain, which may be over-looked or confused in some cases with the neuropathic pain of syringomyelia. It is therefore important for veterinary practitioners to be aware of chronic pancreatitis in cavaliers and to institute appropriate management including analgesics with the aim of reducing the morbidity.

Gastrointestinal clinical signs are commonly reported in Cavalier King Charles Spaniels, affecting 19.3% of dogs in a recent study (Summers *et al.* 2015), and it is possible that this is due to chronic pancreatitis in a significant number of cases, considering the high prevalence in this study. However, 34.6% of the dogs without histopathological evidence of chronic pancreatitis also had clinical signs which could have been consistent with this disease, suggesting that other gastrointestinal diseases are also common in this breed.

A weak, but significant correlation was identified between age and severity of chronic pancreatitis, this suggests that chronic pancreatitis is a progressive disease which worsens with age in this breed.

This is the first study to report the prevalence of renal disease in Cavalier King Charles Spaniels. Glomerulonephritis or interstitial nephritis was identified in 19 dogs (41%) in this study, whilst only 4 dogs had an ante-mortem diagnosis of renal disease. 24 dogs (44.4%) in this study had congestive heart failure and were on long-term medication for cardiac disease and this may mean that subtle changes in renal function were overlooked. Many of the dogs were on medications that may have had an impact on renal perfusion, for example angiotensin converting enzyme inhibitors (ACEi) and diuretics and the assumption may have been that mild azotaemia was due to reduced renal perfusion or the effects of diuresis. However, the changes identified on histopathology were indicative of primary renal disease in many dogs. It is likely that at least some dogs with glomerulonephritis had measurable proteinuria and some dogs with nephritis had poorly concentrated urine. However, the retrospective nature of these samples means that we do not have comprehensive information on indicators of renal function in most dogs, and the standard ante-mortem tests for assessing renal function are relatively insensitive. It is very important to increase recognition of chronic renal disease in CKCS ante-mortem because there is strong evidence that changing dogs on to a phosphate restricted diet formulated for renal disease can increase life-expectancy by a factor of 3 (Jacob *et al.* 2002). However, clinicians also need to be aware that renal diets generally have elevated fat concentrations, which may be problematic if the dog also has chronic pancreatitis. Renal or urinary disorders only affected 4.5% of Cavalier King Charles Spaniels presenting to primary-care practices (Summers *et al.* 2015) suggesting that clinicians may need to be more proactive in screening for these diseases. The very different populations between these two studies likely accounts for some of this difference.

Hepatic lesions were commonly detected in this study but the majority of cases had secondary findings likely to be related to other diseases. The finding of congestion was assumed to be related to chronic heart disease. This was not supported by the relative risk statistics, but sample size was

small. The finding of hepatocyte vacuolation is likely to be a secondary reactive change of the liver to a variety of other diseases. The prevalence of secondary hepatic lesions was very similar to that reported in a previous study in a number of breeds of dogs (Watson *et al.* 2010b).

The authors considered whether an association might exist between the different abnormalities encountered. It was hypothesised that chronic pancreatitis might lead to an increased risk of glomerulonephritis due to the chronic inflammation and potential for immune complex deposition. Alternatively it was thought possible that chronic renal disease might cause pancreatitis, due to alterations in blood flow and an increase in circulating toxins. However in this study there was no increased risk of glomerulonephritis or interstitial nephritis in CKCS with chronic pancreatitis. It was equally considered that vacuolar hepatopathy may occur as a sequel to the chronic disease of chronic pancreatitis, however we found no evidence of an increased risk in this study. This agrees with a previous study (Watson *et al*. 2010b). That study did identify an increased relative risk of reactive hepatitis in dogs with chronic pancreatitis (Watson *et al.* 2010b). Only one dog in the current study was reported with reactive hepatitis so numbers were too small to investigate any potential association.

A significant number of the dogs in this study had been diagnosed with cardiac disease or syringomyelia, a fact that is not surprising due to the high prevalence in this breed. It is possible that this could have had an impact on the other diseases of interest. However, a larger population would be required to explore this possibility further.

There are a number of limitations to this study. The first is the retrospective nature of the study, which meant that the CKCS had been exposed to a number of different therapeutic regimes ante-mortem, which may have impacted the findings. The study was also reliant on owners to provide

accurate clinical information which means that some information may have been missing, and was a self-selected population which was likely to attract owners with an interest in CKCS diseases and potentially dogs with chronic disease. Investigating pancreatitis is also inherently difficult, as the lesions are not always uniformly distributed throughout the organ. Lastly, the grading of pancreatitis is relatively subjective. However all of the pancreas sections in this study were reviewed by one person, which should allow some consistency of interpretation.

In summary, this is the first study to look at the prevalence of microscopic lesions of the liver, pancreas and kidney in the Cavalier King Charles Spaniel and it demonstrates a surprisingly high prevalence of chronic pancreatitis and renal disease in this breed (51.9% and 52.2% respectively), most of which were not diagnosed ante-mortem. The rates of hepatic disease are very similar to the general population. It is hoped that the results of this study will increase recognition and appropriate treatment of chronic pancreatitis and chronic kidney disease in Cavalier King Charles Spaniels, so improving the quality of life of affected dogs. Studies in the future should focus on understanding the reasons for these high disease prevalences in the breed.

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Figure legends

Fig. 1. Normal pancreas from a 10-year-old female CKCS. Most of the section consists of exocrine adenomeres with multiple pancreatic islets (arrows). H&E, bar: 300 μm.

Fig. 2. Mild pancreatitis in a 12-year-old male CKCS, characterised by one area of dissecting fibrosis (F) with lymphocytic infiltrate. H&E, bar: 300 μm.

Fig. 3. Example of moderate chronic pancreatitis in a 9-year-old male CKCS. Note multiple areas of dissecting fibrosis (arrow) with mononuclear inflammatory cells. H&E, bar: 300 μm.

Fig. 4. Marked chronic pancreatitis in a 13-year-old female CKCS, note extensive fibrosis (F) containing lymphocytes surrounding remaining pancreatic tissue (arrow). The fibrosis takes up >50% of the section. H&E, bar: 300 μm.

Fig. 5. Pancreas of a 16-year-old female CKCS with end stage chronic pancreatitis. Most of the pancreatic tissue has been replaced collagenous stroma admixed with blood vessels (S). Only a very small amount of remaining acinar tissue is found (arrow). The pancreas was not grossly visible at post mortem. H&E, bar: 300 μm.

Fig. 6. An example of a kidney of a CKCS with glomerulonephritis and interstitial nephritis (arrows). H&E, bar: 300 μm.